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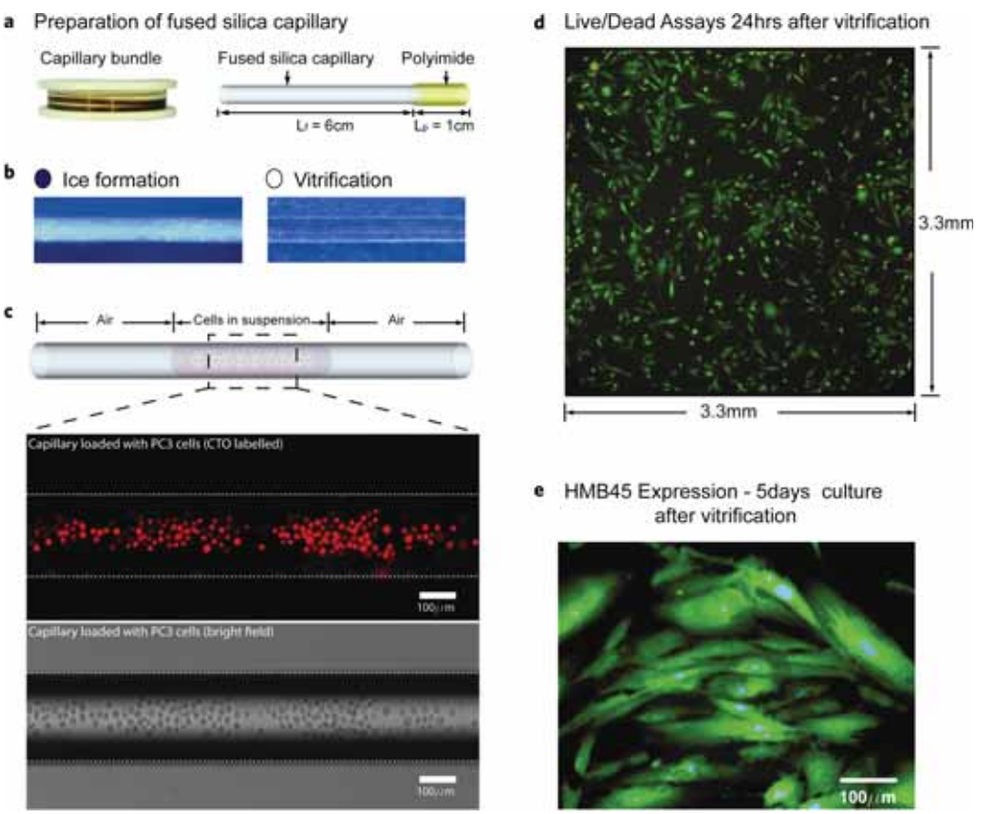
PRESS RELEASE

“Universal” vitrification of cells by ultra-fast cooling



A universal vitrification protocol using silica microcapillaries and low cryoprotectant concentrations was developed. A bundle of capillary with polyimide coating was cut to 7cm length and the polyimide coating was selectively removed by a solvent, N-methylpyrrolidone (a). The performance of the new vitrification protocol can be explained in part by the high cooling rates inside the microcapillaries such as an ice formation and the vitrification of media in the silica microcapillaries with an inner diameter of 200 μm and a wall thickness of 20 μm (b). After re-suspension in the vitrification solution, the cells were then loaded into the 200 μm capillary: A schematic drawing of the loaded cells in capillary (c-top), PC3 labeled with Cell Tracker Orange (CTO) epifluorescence in Red (c-middle), and the bright field image of the cells in capillary (c-bottom). The sets of microcapillary with cells were plunged into slush nitrogen and submerged for ~ 60 seconds. After warming, the cells were cultured in an incubator for 12–24 hours for the viability assay and 4-5 days for the growth rates assay: the viability of cells from LAM patient pleural effusion at 18hrs after vitrification using 200 μm(ϕ)-20μm(t) fused silica capillary (d) and the HMB45 expression in cells from a LAM patient pleural effusion (e). Scale bar for 100μm.

A team of researchers from the Massachusetts General Hospital, Harvard Medical School in Boston, MA have demonstrated a "universal" protocol for cell preservation. The new method is independent of cell type, unlike current state of the art protocols that have to be optimized for each cell type. It also uses only low concentrations of cryoprotectant, which is an advantage when preserving more sensitive cell types.

Long term preservation of live cells is critical for a broad range of clinical and research applications. The cells that need to be preserved are increasingly diverse (e.g. oocytes, stem cells, genetically engineered cells), and current preservation protocols require careful optimization for each cell type. The optimization process is usually tedious and poses significant limitations for all but the most expert users. To address the challenge of long-term storage of various cell types, the authors took the original approach of loading the cells in fused-silica microcapillaries before preservation. When protected by the tiny glass, cells can be cooled ultrafast, with cooling rates as fast as 4,000 K/s. The ultrafast cooling rate is critical for avoiding the cell damage from the ice crystals that form during freezing. With this protocol, the formation of ice crystals is avoided completely, by a process known as vitrification. The microcapillaries are made of highly conductive fused silica, which are commonly used for analytical chemistry and other industrial applications, making them easily accessible for any user. The report appears in the April 2015 issue of the journal TECHNOLOGY.

Cells are a key component for a myriad of biomedical applications ranging from stem cell therapeutics to tissue engineering, and from drug screening to reproductive medicine. To assure off-the-shelf access to cellular therapies, optimized protocols for the long-term preservation of the cells have been on demand but the optimization process becomes practically prohibitive when considering the need to cryopreserve the very heterogeneous range of cells isolated from animals and humans.

Vitrification (or "ice-free" cryopreservation) is an alternative preservation approach to cryopreservation, which uses very high concentration of cryoprotectant cocktails to completely prevent ice formation. Cryoprotectant concentrations as high as 4–8 M are typically required. Because such concentrations are very toxic for most cell types, individual protocols are optimized for each cell type depending on its sensitivity to the cryoprotectant.

To overcome the limitation of current cryopreservation and vitrification protocols, the authors report the development of an assay that can be easily standardized and implemented without changes for a wide variety of cell types. The new vitrification protocol increases the cooling rate (and effectively increasing survival) by an order of magnitude through the use of fused silica microcapillaries. These industrially manufactured microcapillaries have well controlled dimensions and physical properties, allowing them to function as a low-thermal-mass, high-heat conductivity cell container. Additionally, this new vitrification protocol requires low, non-toxic concentration of cryoprotectant solution. The assay was validated using five cancer cell lines, one fibroblast cell line, primary rat hepatocytes, normal human mammary epithelial cells (HMECs), and tumor cells obtained from the pleural effusion of one patient with lymphangioleiomyomatosis (LAM). For all nine types of cells, high yield and high viability after vitrification were achieved.

One important practical aspect of the new vitrification technique is its ease of implementation and reproducibility. Fused silica capillaries are commonly used as analytical chemistry tools for applications such as capillary electrophoresis and chromatography. These capillaries are manufactured under strict quality control for precise diameter, wall thickness, and straightness characteristics. Wider use of the easily available fused silica microcapillaries could eventually lead to standardized cell vitrification protocols across various laboratories, for clinical and research applications.

The team now plans to translate the technology into animal studies, generating xenografts with the vitrified cells. Additionally, the authors are exploring the use of this protocol for the long term banking of oocytes, embryos and rare, delicate clinical samples such as circulating tumor cells.

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